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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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THIS QPR IS BEING SENT TO YOU BEFORE IT HAS BEEN REVIEWED BY THE STAFF OF THE NEURAL PROSTHESIS PROGRAM.

SUMMARY

The pyramidal (corticospinal) tract is a direct, albeit rather sparse, projection from the sensorimotor cortex to the spinal cord. A recording microelectrode implanted chronically in the tract permits monitoring of the neuronal activity induced by an array of stimulating microelectrodes in the sensorimotor (cruciate cortex). Ee have developed a procedure for recording the action potential from individual corticospinal axons via a single small recording electrode.

Using our cat model, we are conducting 3 types of studies that entail recording from the pyramidal tract during intracortical microstimulation with an array of 5 closely-spaced activated iridium microelectrodes (interelectrode spacing of 380-500 μm). These are: (1) Tracking the positional stability of the intracortical microelectrode array, (2) Monitoring the depression of neuronal excitability (SIDNE) induced by prolonged intracortical stimulation, and (3) A comparison of the severity of the SIDNE induced by simultaneous vs interleaved pulsing of the closely-spaced intracortical microelectrodes.

The tracking studies require that we monitor, over an interval of many days, the threshold and recruitment characteristics of the action potential from individual corticospinal neurons. These studies indicate that intracortical microelectrodes that have been implanted for 30 days or more may continue to move slightly within the cortex. However, the thresholds of the corticospinal neurons did remain quite stable over a 20-day interval, and this indicates that the movement is relatively small. At 30 days after implantation there did not appear to be any unidirectional "migration" of the microelectrodes through the cortex.

Other studies demonstrated considerable stimulation-induced depression of neuronal excitability (SIDNE) when 5 closely-spaced microelectrodes are pulsed for 7 hours at a constant frequency of 50 Hz (or more) and at 3 to 4 nC/phase, The SIDNE was somewhat less severe when the 5 electrodes were pulsed in the interleaved mode. When the corticospinal neurons are excited directly, The SIDNE was minimal when the pulsing rate was 25 Hz per microelectrode, when the pulsing was interleaved across the array,

when the stimulus charge per phase was 2.4 to 4 nC/phase. However, these stimulus parameters may induce greater SIDNE if the corticospinal neurons are excited transynaptically.

METHODS

Fabrication of the microelectrode arrays

The shafts of the discrete iridium microelectrodes are made from iridium wire, 35 μm in diameter. One end of each shaft is etched electrolytically to a cone with an included angle of 10° and with a blunt tip approximately 12 μm in diameter. After the tips have been shaped to the proper configuration, a Teflon-insulated wire lead is micro-welded near the upper end of the shaft. The shaft is then insulated with 4 thin coats of Epoxylite electrode varnish, and each layer of insulation is baked using a schedule recommended by the manufacturer.

The insulation is removed from the tip of the shafts by dielectric destruction. The surface area of the exposed tip is determined by measurement of the double-layer capacitance while the tip is emersed in phosphate-buffered saline solution, and using fast (100 Hz) cyclic voltammetry. The measurements are based on a calibration factor relating fast double layer capacitance to electrode surface area (0.55 pf/ μm^2), assuming a constant roughness factor (the ratio of real-to-geometric surface area). The capacitance measurements are performed using a voltage excursion of only 200 mV, in order to avoid activation of the iridium surface during the measurements. In order to standardize the measurements, the surface of the working electrode is "stripped" by holding it at potential at 1.5 V (vs. a saturated calomel electrode) for 5 seconds. The measurements are always performed at -500 mV vs. SEC, since the double layer capacitance is somewhat dependent upon the voltage at which it is measured. In the series described in this report, the geometric surface area was 450 μm^2, +/- 15%

The individual microelectrodes are then assembled into arrays of 7, which extend 1.2 to 1.5 mm from an epoxy matrix. The matrix is 2 mm in diameter. The microelectrodes are then "activated" (a layer of high-valence iridium oxide formed by anodic conversion) by potentiodynamic cycling between -0.8 and +0.7 volts with respect to a saturated calomel electrode, with the microelectrodes immersed in saturated sodium phosphate solution. The activation process is terminated when each microelectrode has a total charge capacity of 200 nC.

Surgical Procedure

Aseptic technique is used during the surgical implantation of the microelectrodes arrays. Young adult cats of either sex are anesthetized initially with Ketamine with transition to a mixture of 70% nitrous oxide, 30% oxygen and 1.5% Halothane. The surgical procedure is carried out with the animal's head in a stereotaxic apparatus. The scalp and temporalis muscle are reflected and, using a Hall bone drill, a craniectomy is made over the left frontal cortex extending into the frontal sinus. The frontal air sinus is partly filled with bone cement.

Prior to implanting the intracortical microelectrode array, a monopolar recording electrode, and its accompanying reference electrode, are implanted in the cat's pyramidal (corticospinal) tract in order to record neuronal activity evoked by the stimulating microelectrodes. The recording electrode is fabricated from 0.25 mm Teflon-insulated stainless steel wire. The exposed area at the tip of the stainless steel electrode wire is approximately 0.1 mm^2. In preparation for implantation, the wire is mounted in a sleeve-type cannula device, which is mounted in a stereotaxic assembly. A small burr hole is cut in the calvarium over the cerebellum, and a small incision is made in the dura. A stimulating macroelectrode (approximately 0.5 mm in diameter) is placed against the dura over the pre- or postcruciate gyrus. This macroelectrode can support a large stimulus current (1 to 2 mA, 150 μsec/ph, at 20 Hz) which excites many corticospinal neurons and thus produces a large compound action potential that can be used to guide the recording electrode into the pyramidal tract. When the tip of the electrode is in the pyramidal tract, the inner introducer is retracted, and the recording and reference electrode are sealed to the skull with bone cement.

In preparation for implanting the microelectrode array into the sensorimotor cortex, the percutaneous connector is mounted to the skull with stainless steel screws and methacrylate bone cement. A small flap, slightly larger than the array's superstructure matrix, is made in the dura over the postcruciate cortex, and the array of microelectrodes is inserted into the cortex by grasping its cable with padded forceps, and a padded probe is used to push the microelectrodes through pia and down into the cortex. The epoxy

matrix and the cables remain above the pia. Some arrays were inserted with the aid of an axial introducer. During the implantation, a vacuum holds the arrays against the orifice of the introducer tool. In our cat model, we have found that it is best not to suture the dura over the array, but we do cover the array with a sheet of perforated artificial dura (silastic sheeting), which rapidly becomes overgrown with dura. The silastic sheet prevents the array from floating out of the cortex. The cortex and silastic disk are covered with Gelfoam and the skull defect is sealed with cranioplasty.

Stimulation protocols

The test stimulation protocols were conducted at least 45 days after the implant surgery. During the stimulation, the cats were lightly anesthetized with Propofol. We have determined that the electrical excitability of the corticospinal neurons is not altered by light Propofol anesthesia, and the cats are much easier to manage when lightly anesthetized than when awake and wearing the tethered backpack.

In each array, 5 of the 7 microelectrodes were pulsed continuously for 7 hours. The microelectrodes were pulsed either simultaneously or sequentially (interleaved stimulation). The stimulus was charge-balanced, controlled current biphasic pulse pairs, 150 μs/phase (cathodic phase first). The activated iridium microelectrodes were biased at +400 mV with respect to the implanted Ag/AgCl reference electrode.

Within 30 minutes after the end of the stimulation regimen, the cats were deeply anesthetized with pentobarbital and perfused through the aorta with ½ strength Karnovsky's fixative (2.5% glutaraldehyde, 2% paraformaldehyde and 0.1M sodium cacodylate buffer). The array of microelectrodes was removed from the cerebral cortex, after resection of the overlying connective tissue. The block containing the array tracks was resected, embedded in paraffin, sectioned serially in the horizontal plane (perpendicular to the shafts of the stimulating microelectrodes) at a thickness of 8 μm, and stained with Cresol Violet (Nissl stain).

RESULTS

In this report, we present data from 3 ongoing studies that entail recording from the pyramidal (corticospinal) tract. These are: (1) Tracking the positional stability of the intracortical microelectrode array, (2) Monitoring the depression of neuronal excitability (SIDNE) induced by prolonged intracortical stimulation, and (3) A comparison of the severity of the SIDNE induced by simultaneous vs interleaved pulsing of closely-spaced intracortical microelectrodes.

Stability of the Intracortical Array.

Figure 1 shows a family of responses recorded from the pyramidal tract of cat IC161 while pulsing microelectrode #7 of the intracortical array. Since only a single electrode is used to record action potentials from the entire pyramidal tract, the signal-to-noise ratio of the recorded neural activity may be very low. To compensate for this, 4096 repetitions of the stimulus were summated to obtain each trace. The abscissa is the time after the stimulus pulse. The values near the right margin of the figure, and above each trace, indicate the amplitude of the stimulus pulse that was used to evoke each response. The stimulus pulse duration was 150 or 400 μsec/phase. A neuronal response with a latency of 2.2 msec after the stimulus is present in each trace for which the stimulus amplitude was 8 μA or greater. This response component is enclosed by the ovoid. Because of its short duration and its near "all-or-none" character, we conclude that this response component represents the action potentials from a single corticospinal axon.

Figure 2 shows the response growth, or "recruitment" curves that were obtained by plotting the peak-to-peak amplitude of the component against the stimulus pulse amplitude that was used to evoke the response. The curves were obtained over an interval of 20 days, beginning 30 days after implantation of the microelectrode array. The recruitment curves are graded over a portion of the stimulus range, and ordinarily, we would not expect this to be the case for a response generated by a single neuron (axon). However, the recruitment curves were generated from traces obtained by summating 4,096 consecutive responses. When the stimulus amplitude is close to a neuron's threshold, the

neuron will not generate an action potential in response to every stimulus pulse, and when the action potentials do occur, they will exhibit some temporal dispersion. These two phenomena will cause the amplitude of the averaged response to be graded over a portion of the stimulus amplitude range.

The recruitment curves of the component did vary somewhat over the course of the 20 days. At 30 days after implantation, the neuron's response threshold was no greater than 8 μA, and the amplitude of the response decreased somewhat at high stimulus current. The latter phenomenon probably reflects a partial block of the action potentials by (hyperpolarizing) stimulus current flowing outwards through the cell's axon, at a site that is some distance away from the microelectrode (Ranck, 1975). This anodic block phenomena, and the very low threshold of the response both suggest that at day 30, the intracortical microelectrode was very close to the neuron. By day 42, the neuron's threshold had increased to 10-12 μA, and the anodic block is not evident. This suggests that the neuron was now more distant from the microelectrode. By day 46, the component's threshold had again decreased to below 8 μA, but by day 50, the threshold had increased again.

Figure 3 shows the averaged responses recorded in the pyramidal tract while pulsing microelectrode #5 from the same animal, in this case at 50 days after implantation of the array. Two components can be discerned, one with a post-stimulus latency of 1.1 msec and another with a latency of 1.8 msec. Figures 4A and 4B show the recruitment curves of these components. Here again, the components' threshold varied somewhat over the 20-day interval, but in all cases, the threshold remained below 10 μA. These data suggest that the tip of the intracortical stimulating microelectrode was moving slowly through the tissue, but that the movement was somewhat random, since the thresholds did not change systematically over the 20-day interval.

Figure 5 shows the recruitment curve of a component recorded from the pyramidal tract of cat IC-167 while pulsing intracortical microelectrode #5. Between days 97 and 106 after implantation, the unit's electrical threshold remained essentially constant, at approximately 8 μA. These data are consistent with our other studies that entail recording

action potentials from single cortical neurons via these same intracortical microelectrodes. (Liu et al, 1997) which also suggest that these intracortical arrays do not become positionally stable until at least several weeks after implantation

Stimulation-induced depression of neuronal excitability.

Our previous studies have described the phenomenon of stimulation-induced depression of neuronal excitability (SIDNE) for microstimulation in the feline cochlear nucleus (McCreery et al, 1992, 1997) and in the feline cerebral cortex (McCreery et al, 1986). The results reported here are a part of our effort to determine the relation between SIDNE and certain stimulus parameters for intracortical microstimulation with many closely-spaced microelectrodes (stimulus pulsing rate, stimulus pulse amplitude, stimulus charge per phase).

Figure 6 shows the response recorded from the pyramidal tract of cat IC167 while pulsing intracortical microelectrode #7. There is a component with a threshold of 8 μA or less and a latency of 1.9 msec after the stimulus. At a stimulus amplitude of 13 μA, a second component with a slightly longer latency (2.6 msec) was recruited. Figures 7A and 7B show the recruitment curves for each of these components. The data were acquired before the 7-hour stimulus regimen, immediately after the regimen, and 48 hours later. The stimulus regimen entailed pulsing 5 of the intracortical microelectrodes (including microelectrode #5) for 7 hours at 50 Hz. The pulse duration was 150 μsec/phase. The stimulus pulse amplitude was 26.5 μA (4 nC/ph). The microelectrodes were pulsed in the interleaved mode. Immediately after the 7 hours of stimulation, the threshold of both components was elevated to above 20 μA, indicating severe SIDNE. At 48 hours after the end of the stimulation regimen, the excitability of the neuron depicted in Figure 7B had recovered nearly completely, while the excitability of the neuron depicted in Figure 7A had only partially recovered. The neuron depicted in Figure 7B probably was farther from the stimulating microelectrode (its threshold initially was higher), and this may account for its more complete recovery by 48 hours after the end of the stimulation. However, SIDNE of

this severity would clearly be unacceptable for a neural prosthesis based on microstimulation.

Figure 8 shows the recruitment curves of a component recorded from the pyramidal tract of cat IC154 while pulsing intracortical microelectrode #7. In this animal, the 7-hour stimulus regimen was similar to that used in cat IC167, but the pulsing rate was only 25 Hz per electrode, rather than 50 Hz as in IC167. This regimen induced only a small amount of SIDNE. Our Interpretation of this data is complicated somewhat by the fact that the component initially had a rather high threshold (approximately 10 μA), and this suggests that the neuron was not particularly close to the stimulating microelectrode.

Figure 9A shows the recruitment curves for a component recorded from the pyramidal tract of cat ic166 while pulsing intracortical microelectrode #7. The short latency of this component (1 ms after the stimulus pulse) indicates that the corticospinal neuron was excited directly by the intracortical stimulation. Five of the microelectrodes were pulsed for 7 hours at 25 Hz, in the interleaved mode. The stimulus pulse amplitude was 16 μA (2.4 nC/phase), which was approximately twice the threshold of the component before the start of the stimulation. After the stimulation, the component's threshold was only slightly elevated, indicating minimal SIDNE. Figure 10b shows the recruitment curves for another component also evoked from the intracortical microelectrode #7. The long latency of this component (3.8 ms after the stimulus pulse) indicates that the corticospinal neuron was excited transynaptically by the intracortical stimulation. In this case, the SIDNE was much greater than in the case of the component from the directly-excited neuron (Figure 9A). Thus, in the feline sensorimotor cortex, the prolonged intracortical stimulation may more strongly depress transsynaptic excitation.

The data indicate that stimulating at 25 Hz and at a moderate stimulus amplitude (2.4 to 4 nC/phase) may not induce severe SIDNE of directly-excited corticospinal neurons. Additional studies will be necessary to determine if the severity of the SIDNE is related to the average rate of intracortical stimulation, as appears to be the case for microstimulation in the feline cochlear nucleus (McCreery et al, 1997). If this indeed is the case, then an average stimulation rate of 25 Hz (e.g., 50 Hz with a 50% duty cycle, 75 Hz

with a 33% duty cycle) may be sufficient to implement a neural prosthesis based on intracortical microstimulation.

Effect of Interleaved vs Simultaneous Pulsing.

The tips of the microelectrodes comprising the intracortical array are close together (380-500 μm apart), and the stimulus current field will overlap considerably when adjacent microelectrodes are pulsed. We have compared the severity of the SIDNE that is induced by simultaneous and also by interleaved pulsing of 5 microelectrodes of the intracortical array. The experimental design is depicted in Figure 10. The protocol was initiated 50 days after implantation of the array into cat IC158. The neural activity was recorded from the pyramidal tract before the start of the 7 hours of stimulation, immediately after the 7-hour regimen, one hour after, 15 hours after, and 4 days after the end of the stimulation. In the first 7-hour stimulation, 5 microelectrodes (#3, 4, 5, 6, 7) were pulsed simultaneously at an amplitude of 20 μA (3 nC/ph) and at a pulse rate of 100 Hz. Twelve days later, the same regimen was repeated except that the same 5 microelectrodes were pulsed in the interleaved mode.

Figure 11A shows plots of the threshold of 2 components recorded from the cat's pyramidal tract while pulsing intracortical microelectrode #6. The components had latencies of 1.2 msec and 2.2 msec after the stimulus pulse. In this case, "threshold" is defined as the minimum stimulus current in the recruitment sequence that was able to evoke the component. For example, in Figure 6, the "threshold" is 8 μA. The plots show that 7 hours of simultaneous pulsing of 5 microelectrodes induced a greater increase in the component's threshold (more SIDNE) than did interleaved stimulation. For both modes of stimulation, the threshold remained elevated for at least 4 days after the end of the 7-hour regimen. Figure 11B shows plots of the threshold of a component recorded while pulsing microelectrode #4. Here again, interleaved stimulation induced less (but still considerable) elevation of the component's threshold.

It is not entirely clear why interleaved pulsing should induce less SIDNE, since the current field in the immediate vicinity of the microelectrodes is essentially the same for

interleaved and simultaneous pulsing. However, simultaneous pulsing will excite more neurons, since the pulses from several microelectrodes can summate to produce a supra-threshold stimulus at locations that are some distance from the individual microelectrodes. Thus, SIDNE may, at least to some degree, be related to the totality of the induced neural activity in and around the array, a type of "mass action" phenomenon. We have found this to be the case in the feline cochlear nucleus.

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IC161 30 days after implantation

Response elicited from Electrode 7 (average of 4096 responses)

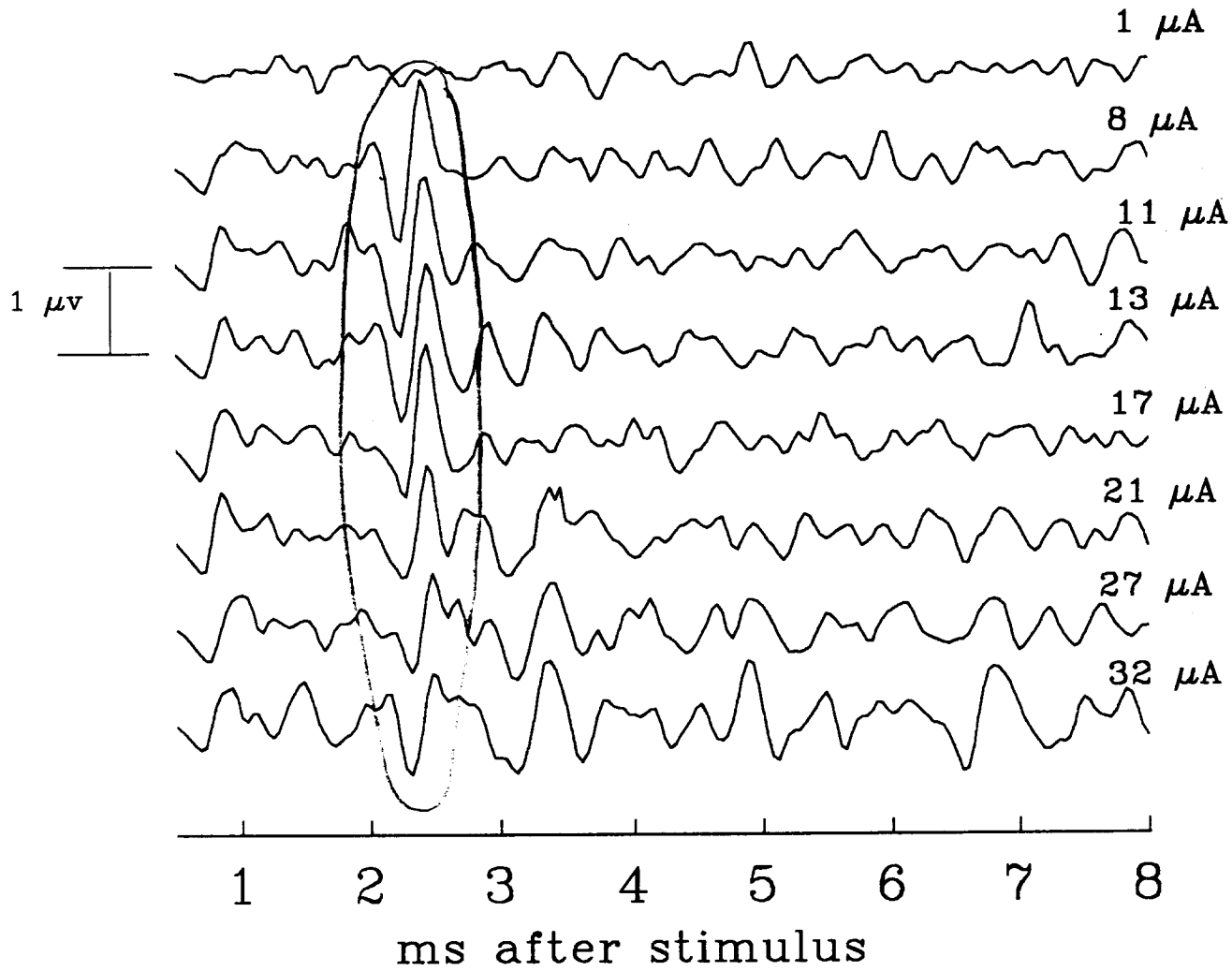

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Figure 1

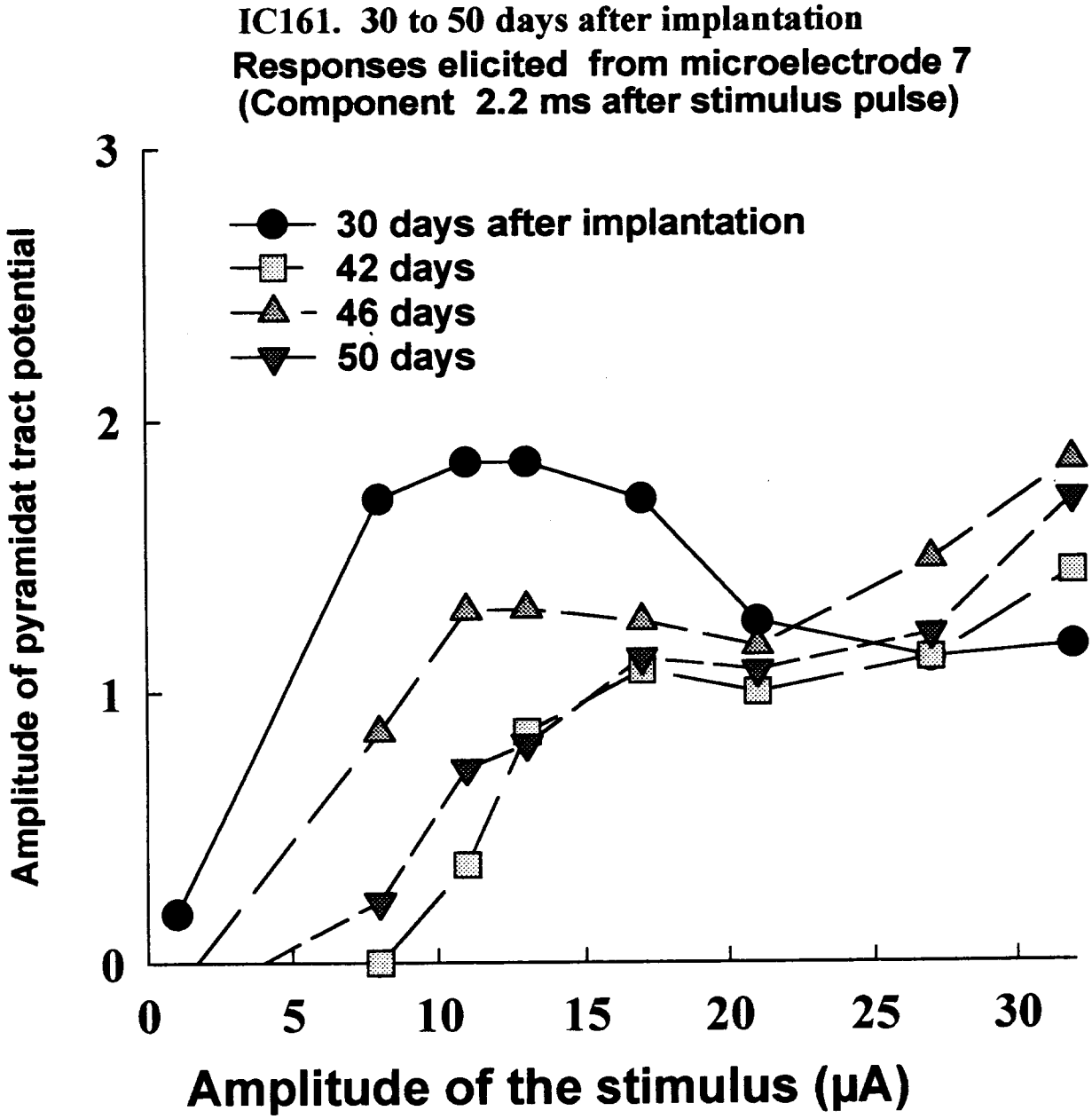

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Figure 2

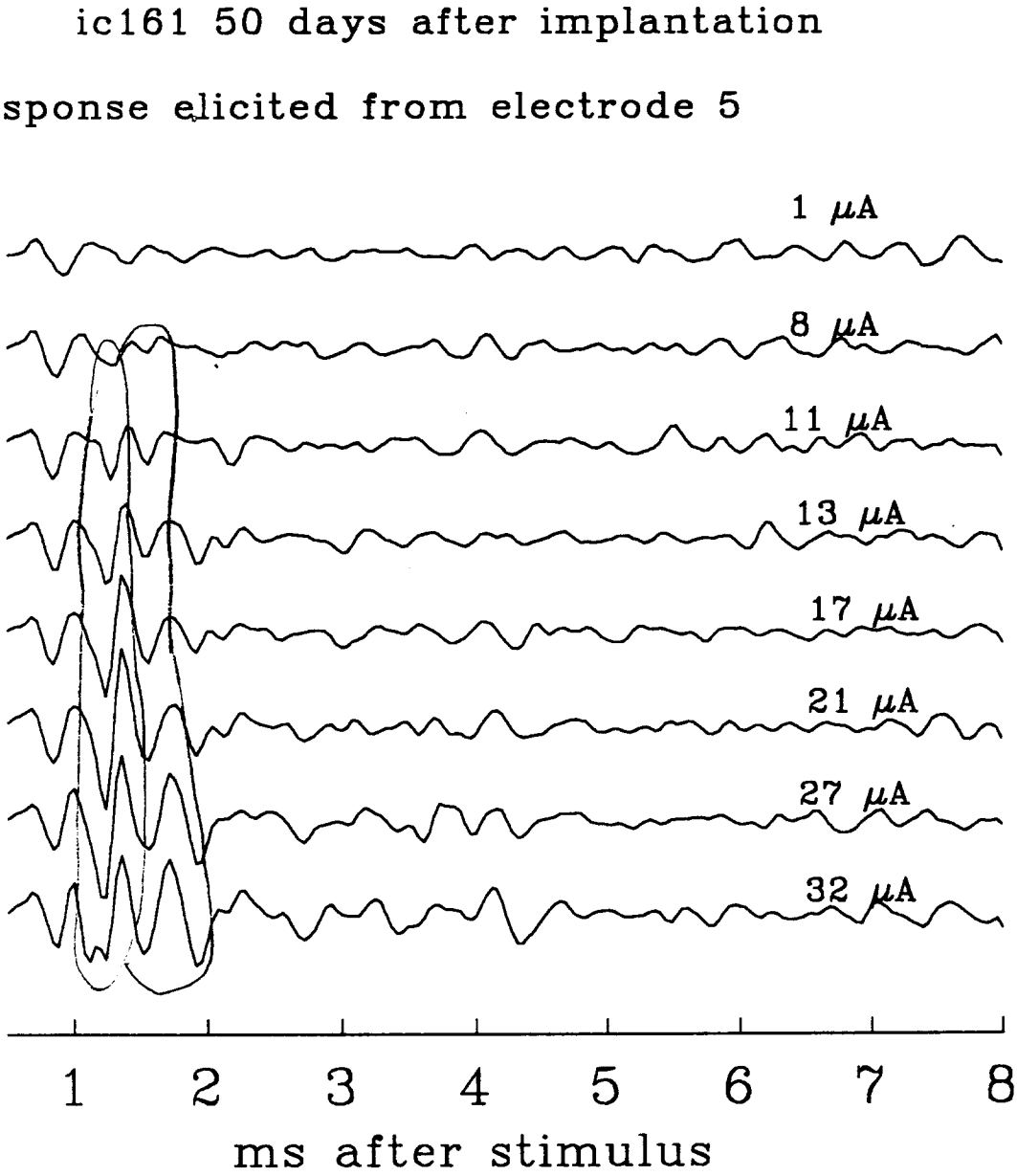

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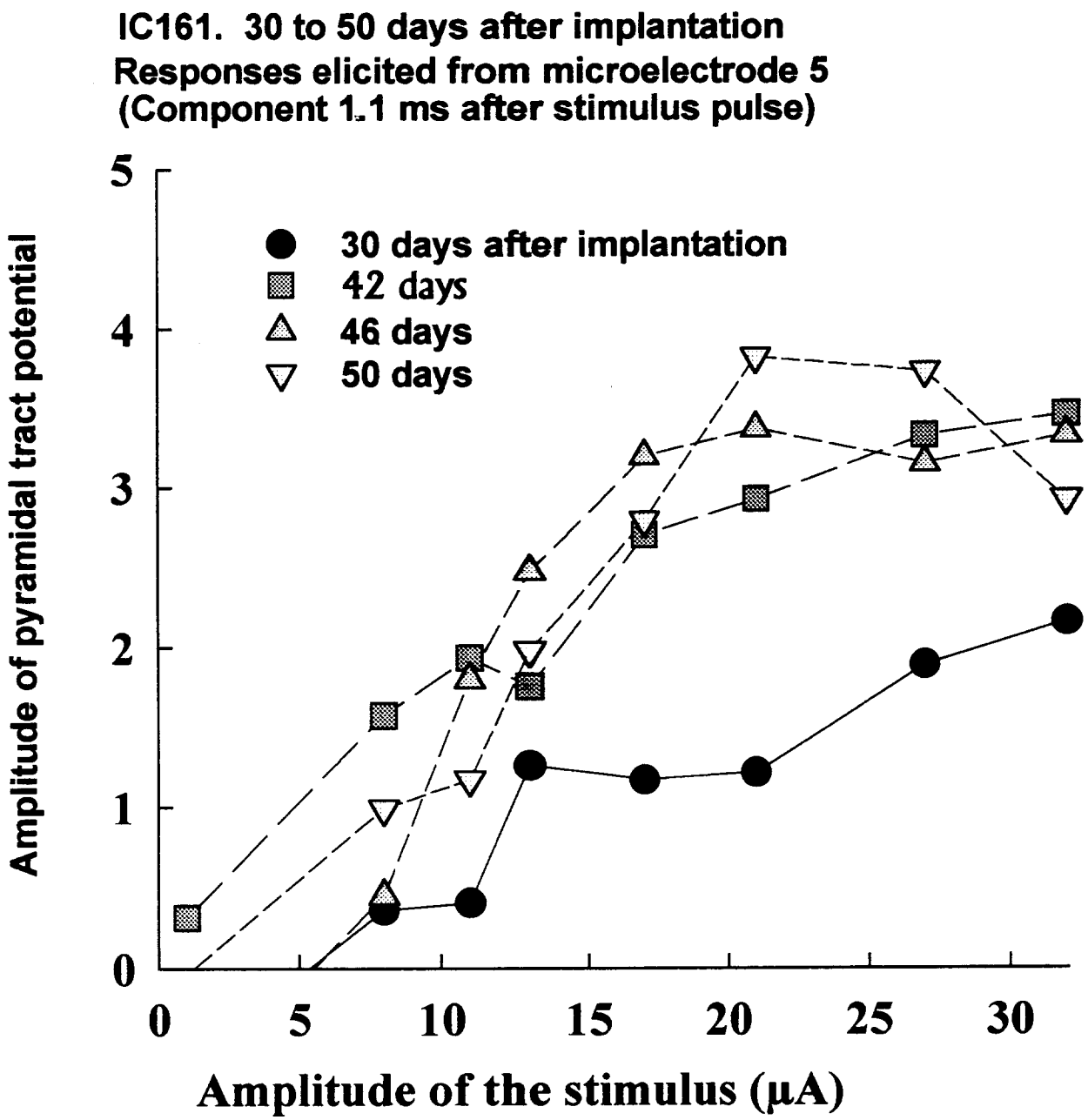
Figure 3



161u5a.spw

Figure 4A

IC161. 30 to 50 days after implantation of array responses elicited from microelectrode 5 (Component 1,8 ms after stimulus pulse)

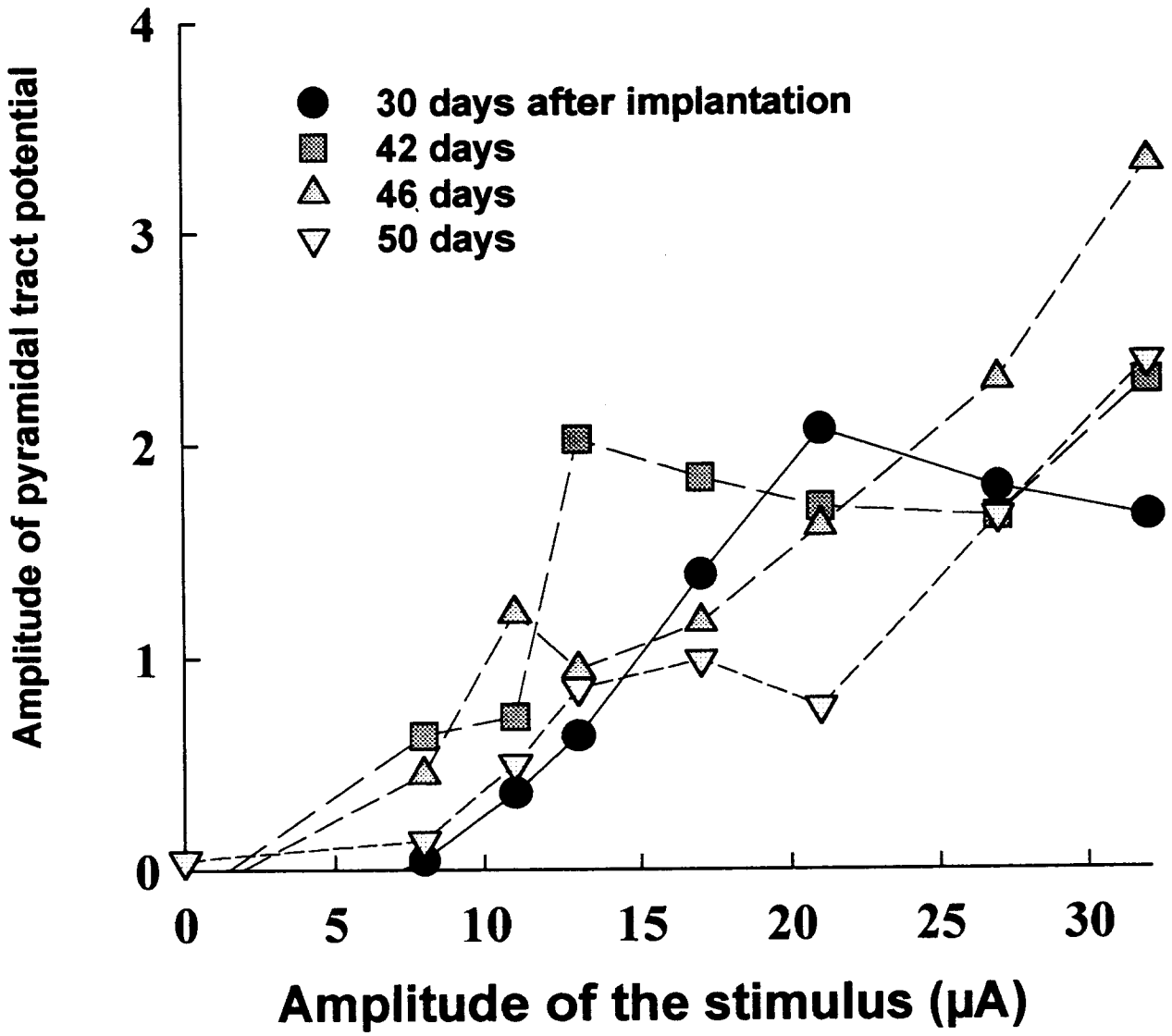


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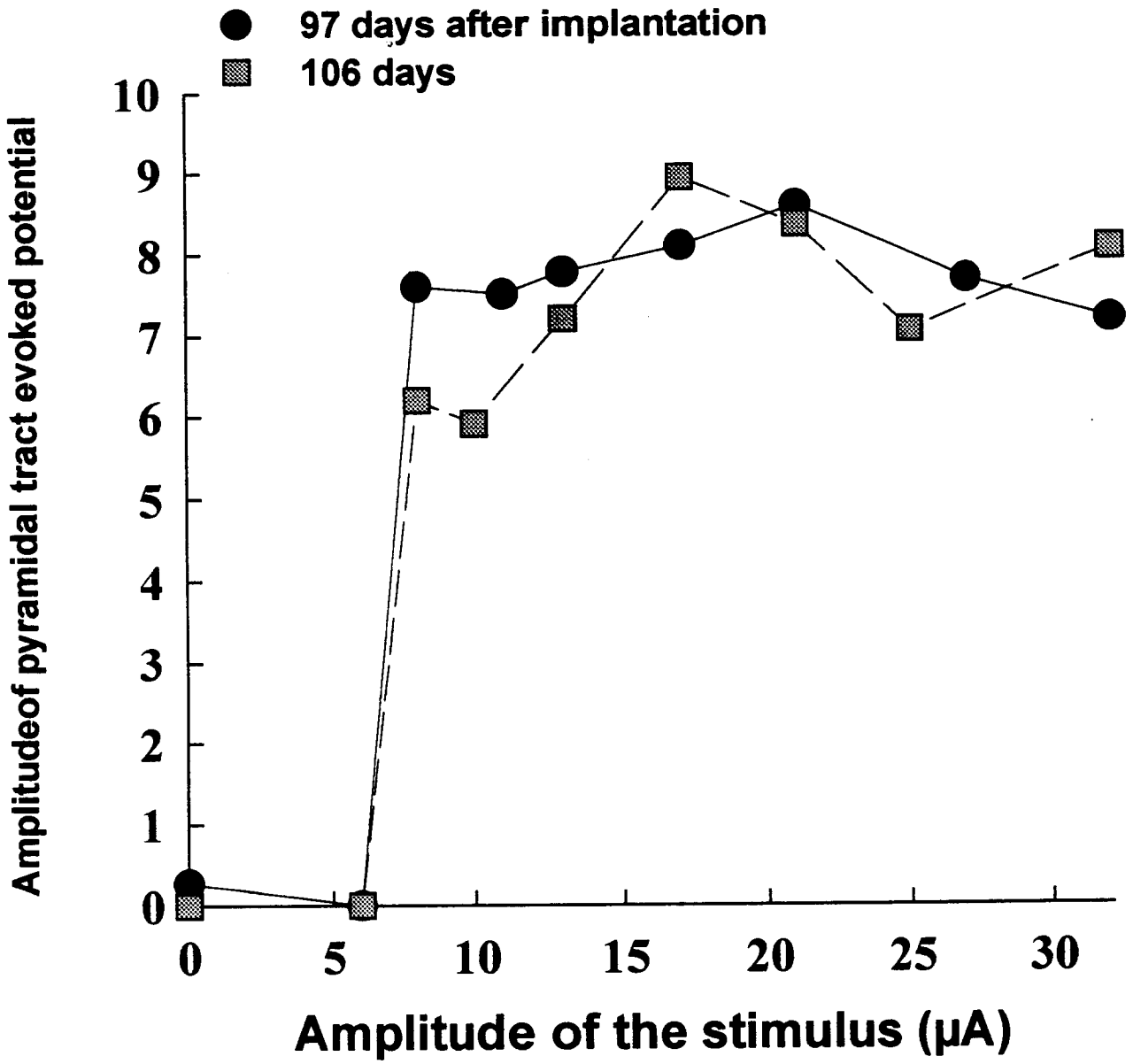
Figure 4B

ic167, 97 to 106 days after implantation of the array
Responses elicited from microelectrode 5

Component 1.96 ms after the stimulus



ic167d.spw

Figure 5

ic167, 106 days after implantation

Responses elicited from electrode 7

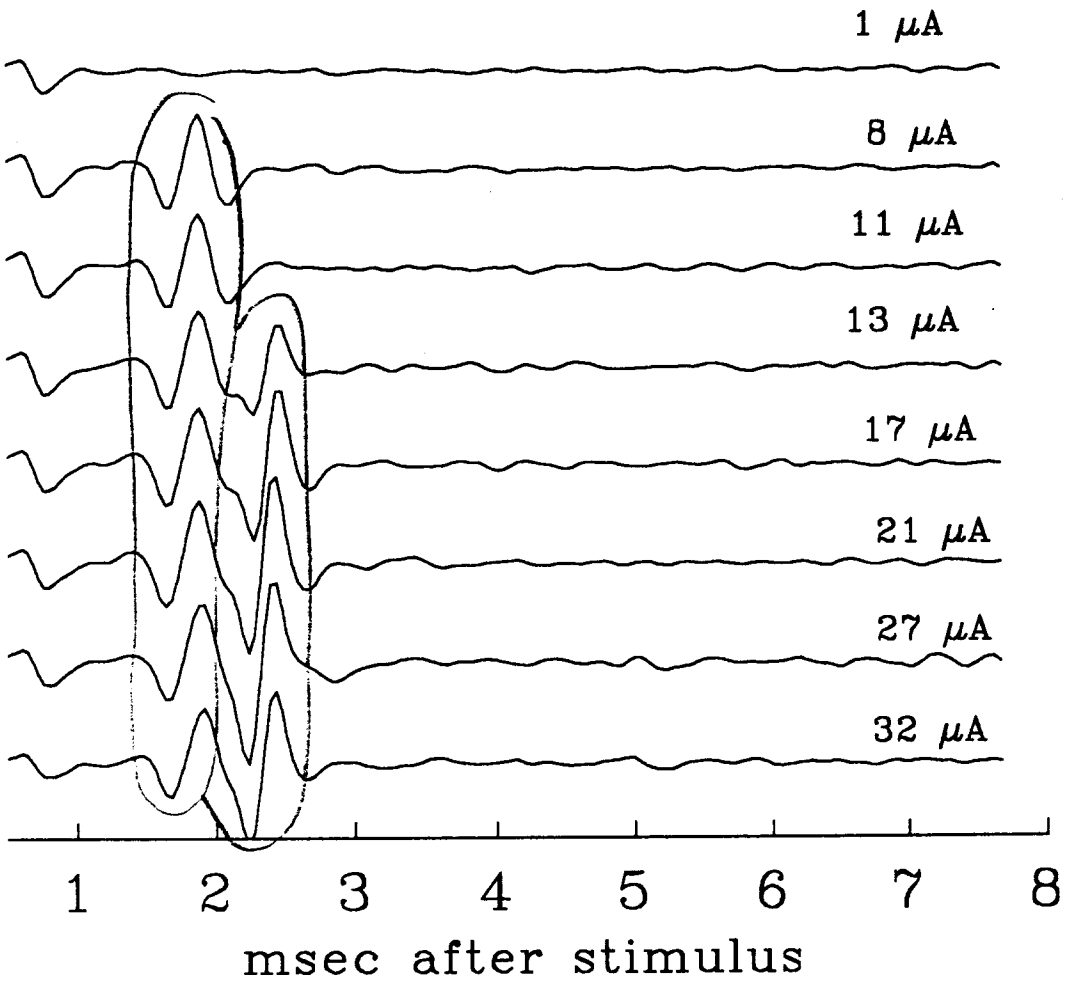


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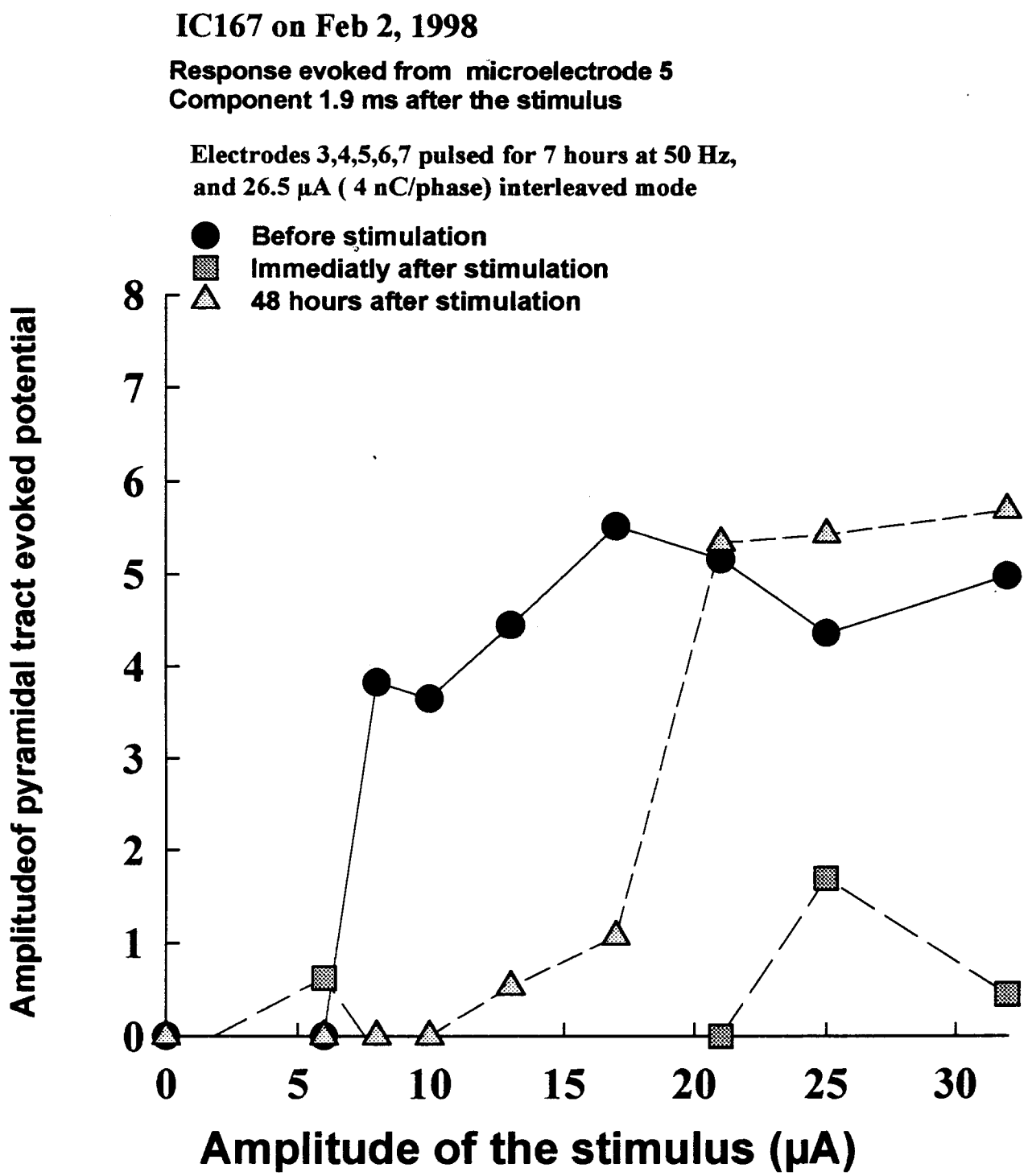
Figure 6



ic167g.spw

Figure 7A

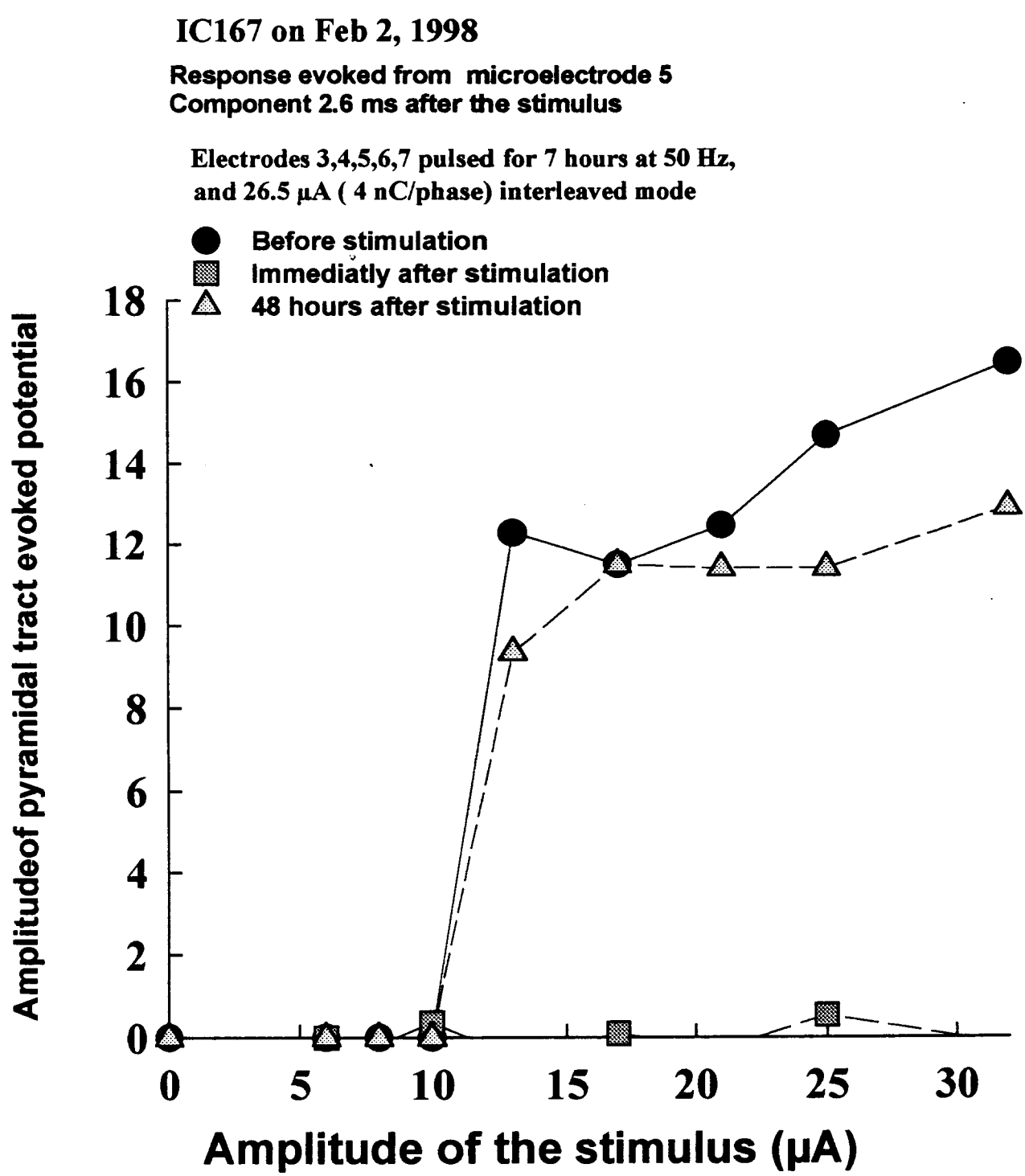


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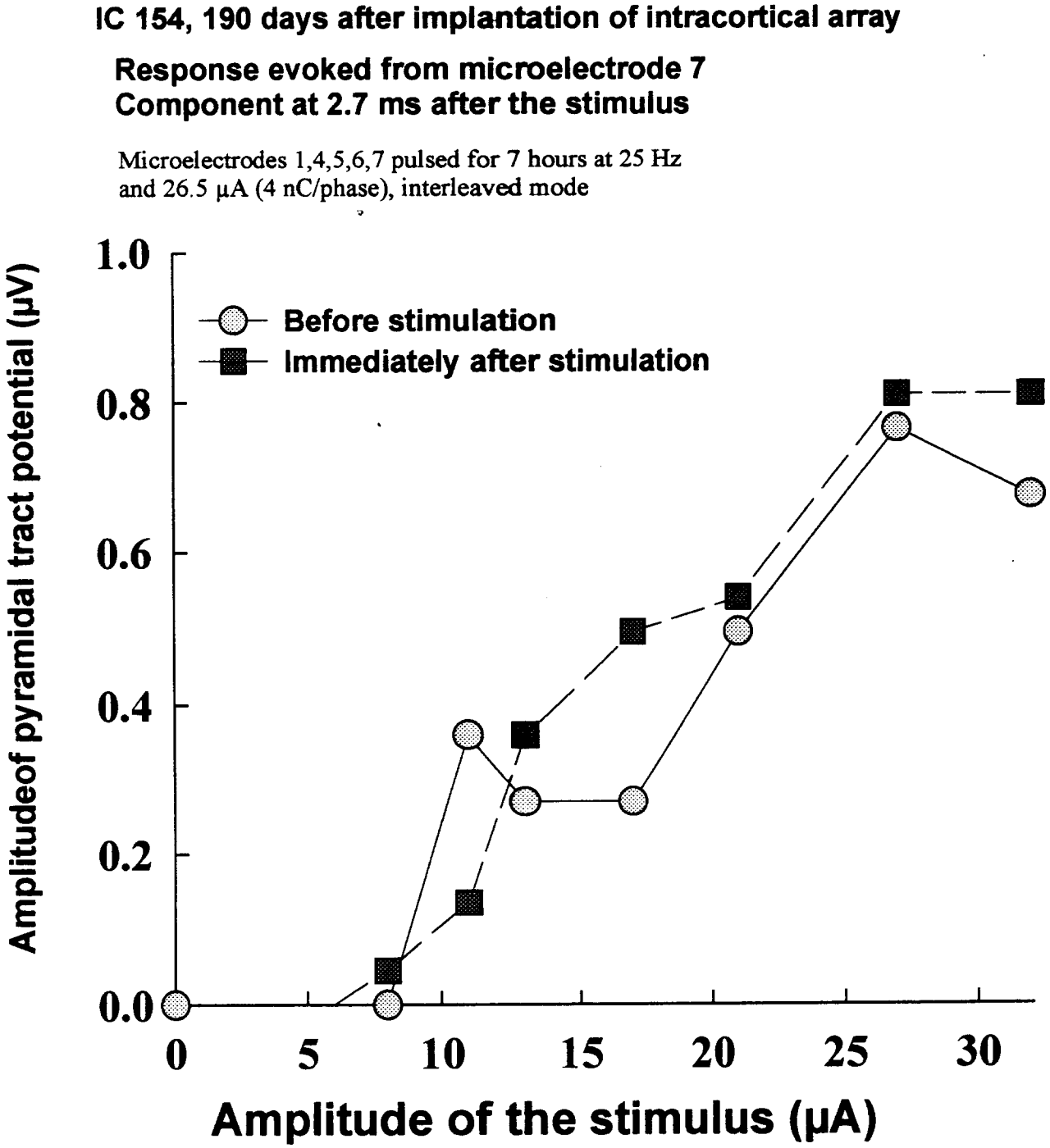
Figure 7B



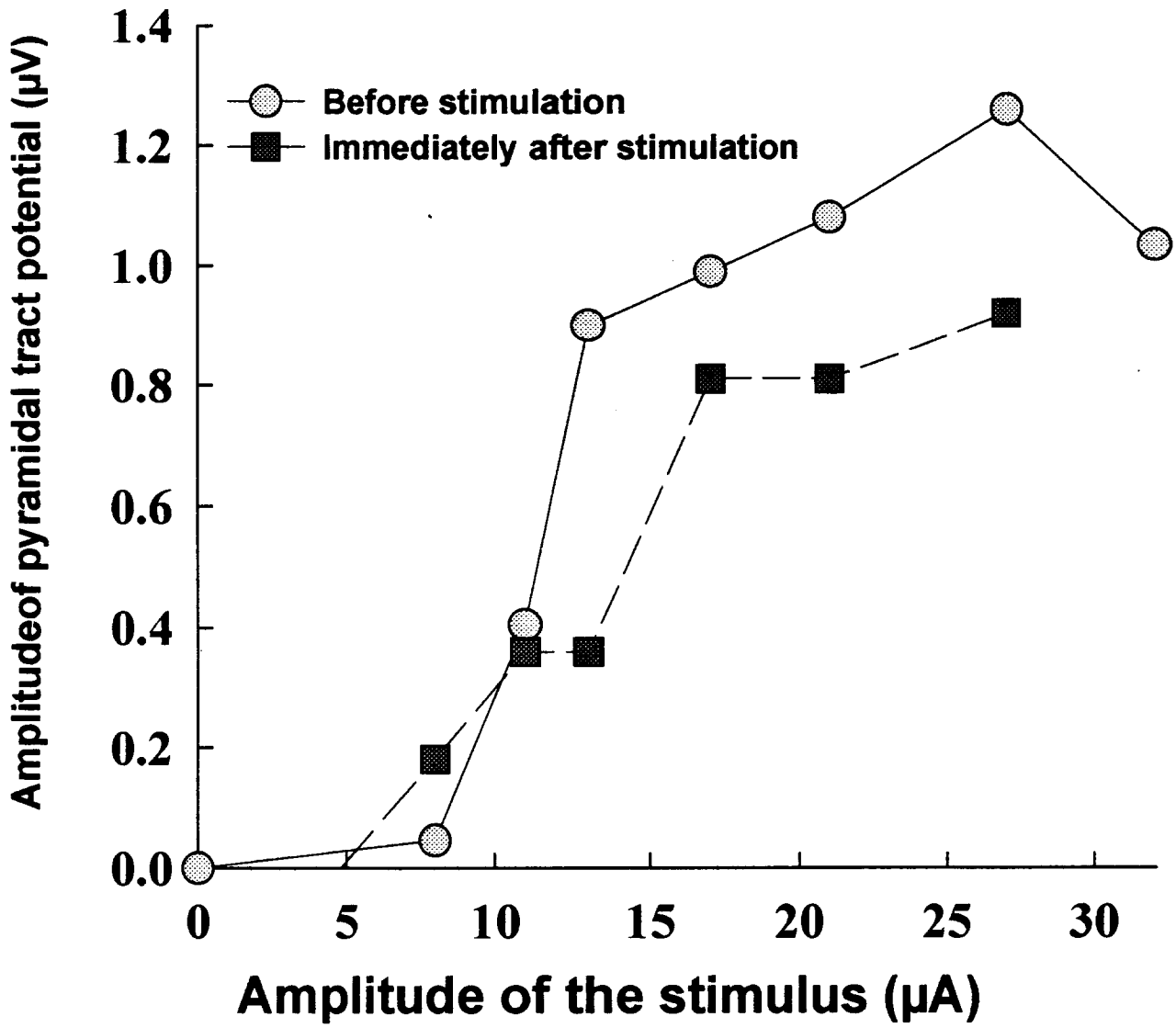
ic154a.spw

Figure 8

IC 166, 48 days after implantation of intracortical array

Response evoked from microelectrode 7
Component at 1.0 ms after the stimulus

Microelectrodes 3,4,5,6,7 pulsed for 7 hours at 25 Hz
and 16 μA (2.4 nC/phase), interleaved mode



ic166a.spw

Figure 9A

IC 166, 48 days after implantation of intracortical array

Response evoked from microelectrode 7
Component at 3.8 ms after the stimulus

Microelectrodes 3,4,5,6,7 pulsed for 7 hours at 25 Hz
and 16 μA (2.4 nC/phase), interleaved mode

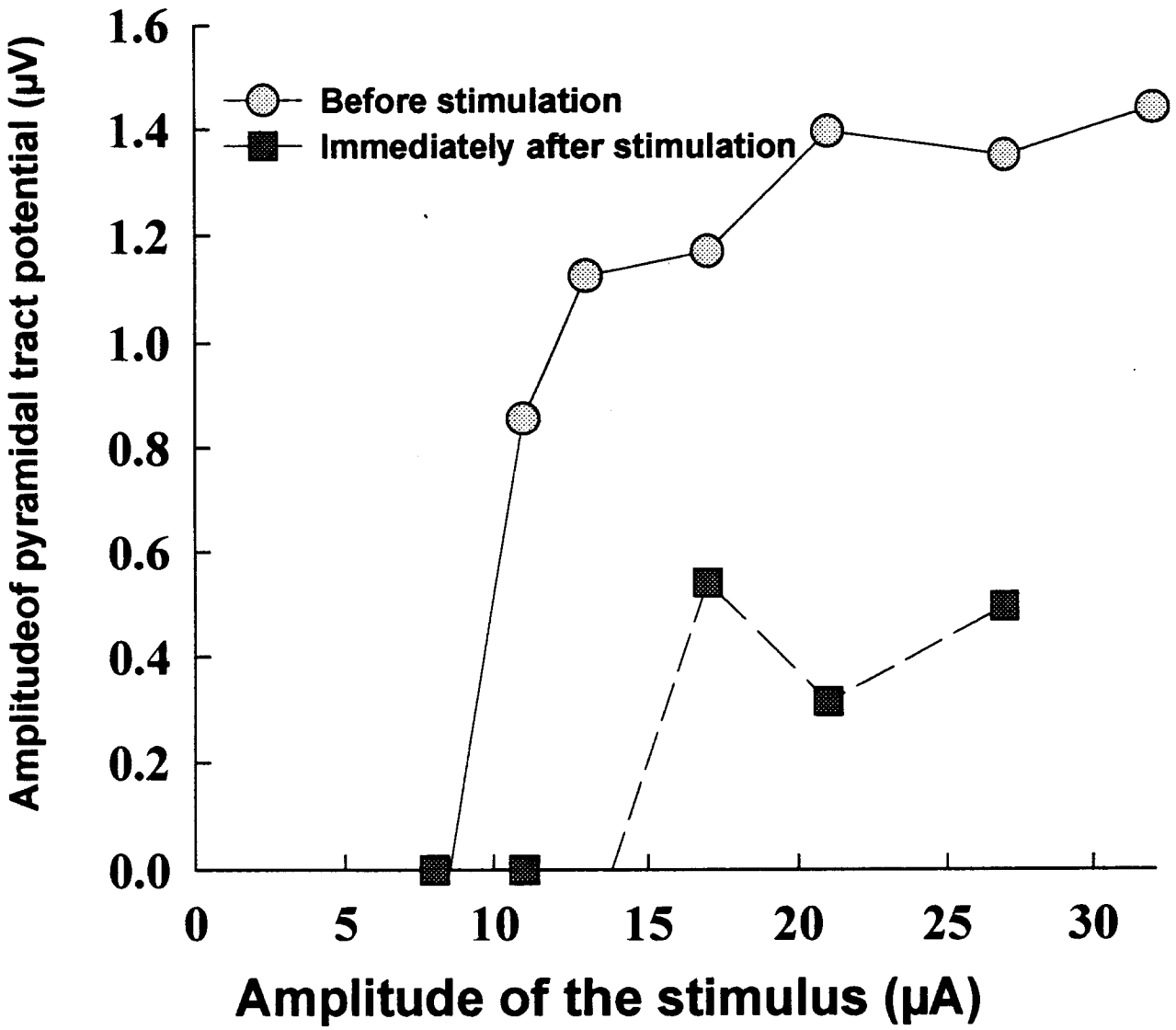


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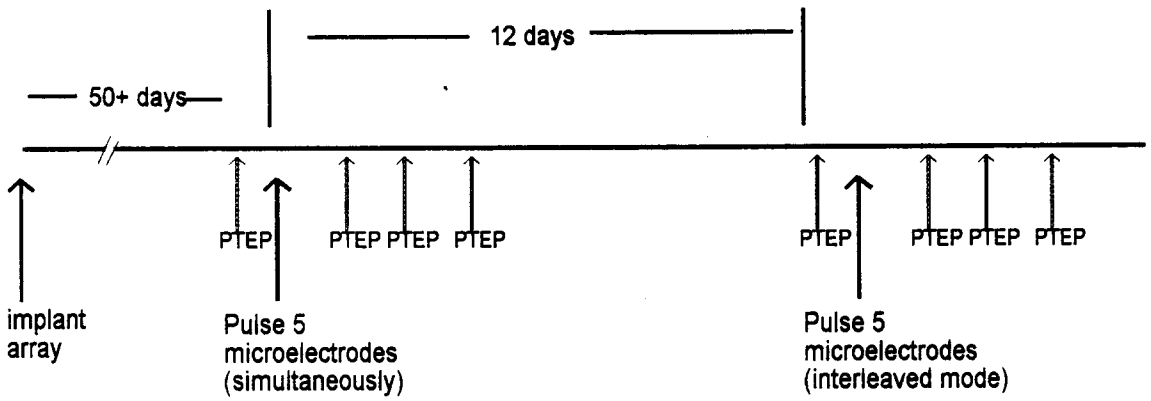
Figure 9B



PTEP = record pyramidal tract evoked potentials

Stimulus parameters
100 Hz per electrode, biphasic current pulses,
150 µsec/phase, 20 µA (3 nC/phase)
for 7 hours

simint.skd

Figure 10

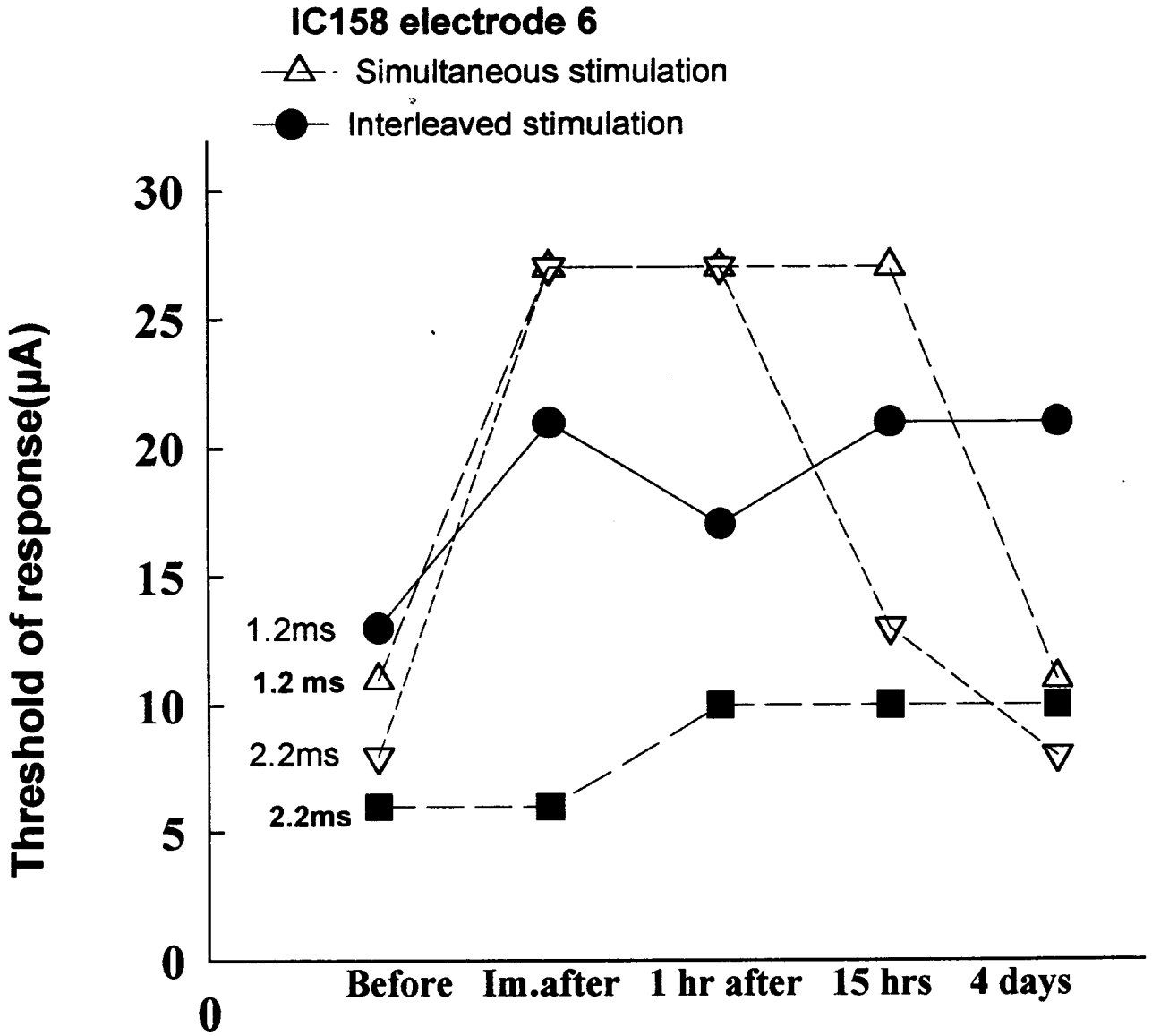


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Figure 11A

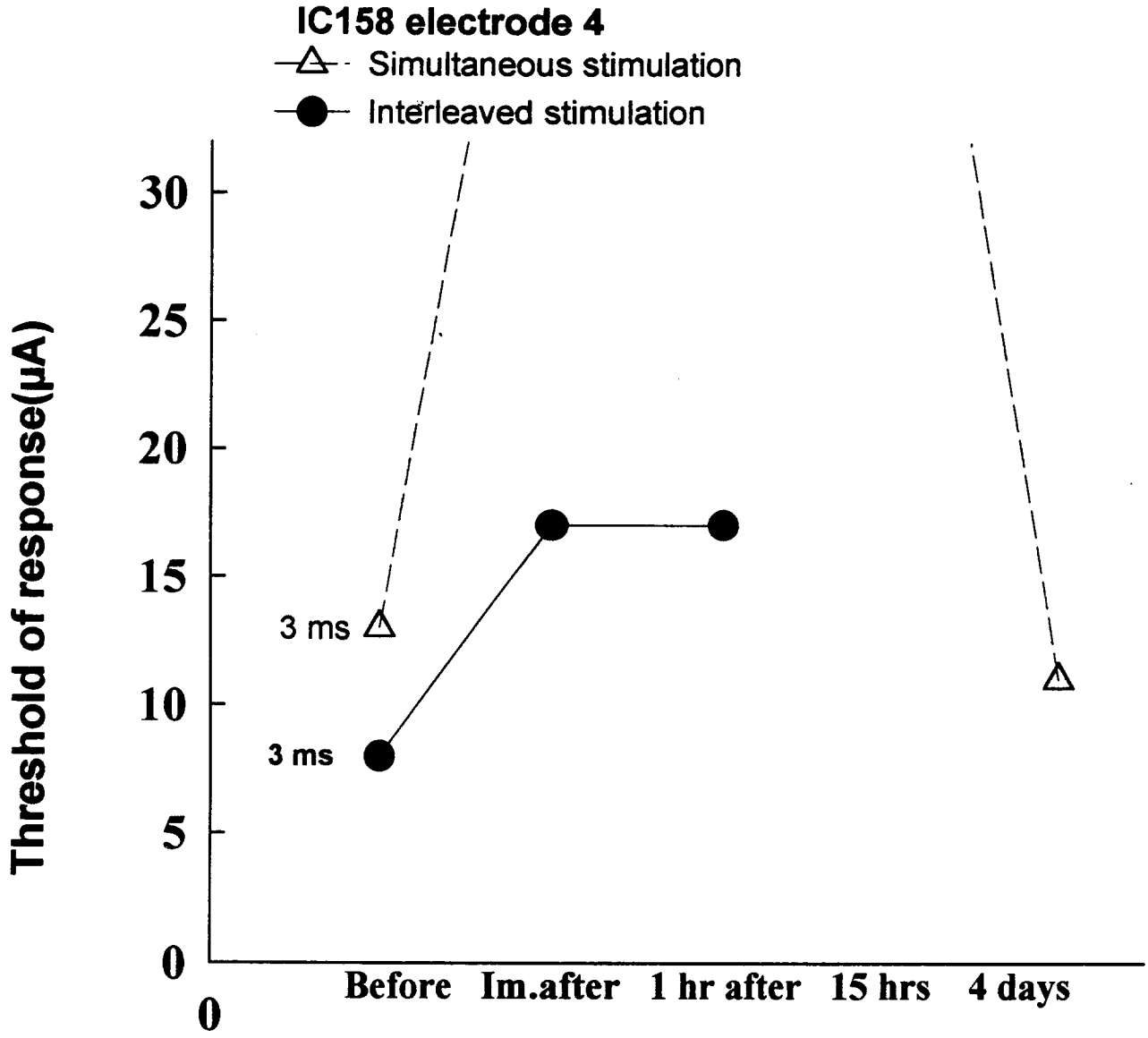

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Figure 11B